ESTROM LDL Cholesterol - Direct
(Homogeneous Direct Method)

ANGSTROM BIOTECH PVT. LTD

INTENDED USE: Kit for the quantitative determination of LDL-Cholesterol concentration in human serum and plasma by direct method

Summary:
Plasma lipoproteins are spherical particles containing varying amounts of cholesterol, triglycerides, phospholipids and proteins. The phospholipid, free cholesterol and protein constitute the outer surface of the lipoprotein particle, while the inner core contains mostly esterified cholesterol and triglycerides. These particles serve to solubilize and transport cholesterol and triglycerides in the bloodstream. The relative proportions of protein and lipid determine the density of these lipoproteins and provide a basis on which to begin their classification. These classes are: chylomicrons, very-low density lipoprotein (VLDL), lowdensity lipoprotein (LDL) and high density lipoprotein (HDL). Numerous clinical studies have shown that the different lipoprotein classes have very distinct and varied effects on coronary heart disease risk. The studies all point to LDL cholesterol as the key factor in the pathogenesis of atherosclerosis and coronary artery disease (CAD) , while HDL cholesterol has been observed to have a protective effect. Even within the normal range of total cholesterol concentrations, an increase in LDL cholesterol can occur with an associated increased risk for CAD.

Principle:
The Estrom LDL-C assay is a homogenous method for directly measuring LDL-C concentrations in serum or plasma, without the need for any off-line pretreatment or centrifugation steps. The method is in a two reagent format and depends on the properties of a unique detergent. This detergent (Reagent1) solubilizes only the non LDL lipoprotein particles(HDL, VLDL,CM). The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non color forming reaction. A second detergent (Reagent 2) solubilizes the remaining LDL particles and a chromogenic coupler allows for color formation. The enzyme reaction with LDL-C in the presence of the coupler produces color that is proportional to the amount of LDL cholesterol present in the sample.

Reagent Presentation:
Reagent 1

Pipes Buffer (pH 7.3),Detergent 1 <4.5%,Cholesterol esterase >2000 U/L,Cholesterol Peroxidase >5000 U/L,100 mMol/L,4-Aminoantipyrine <0.123%

Reagent 2

Pipes Buffer (pH 7.3),Detergent 2 >1.0%,N,N-bis (4-sulfobutyl) <1.0 mM,-m-toluidine, disodium,(DSBmT),Preservative 0.1%,50 mMol/L 0.1%

LDL C Calibrator : 0.5 ML
(Calibrator needs to be reconstituted with 0.5 ml distilled water).
Let it stand for 30 minutes at room temperature .Dissolve the content of the vial by swirling gently to avoid the formation of foam.

Reconstituted calibrator is stable only 7 days at 2-8ºC.

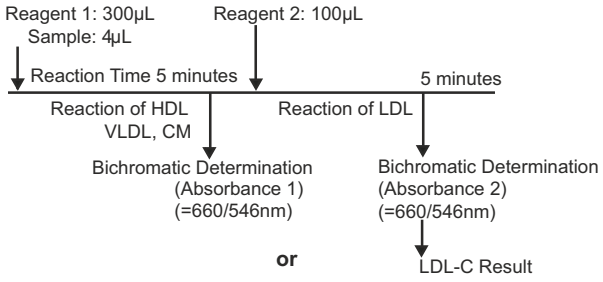
{Estrom LDL-C calibrator must not be cross compared with other commercially available calibrators as all the calibrator manufacturers fix the calibrator concentration depending upon the type of LDL-Cholesterol Assay they are using for calibration of calibrators at their end}.

Storage and Stability:
All unopened reagents are stable until the expiration date on the label when stored at 2-8°C.
Specimen Collection and Preparation:
Patients are not required to fast prior to blood collection.Serum, EDTA-treated or heparinized plasma are the recommended specimens. Anticoagulants containing citrates should not be used because of the possible assay errors due to citrates.If not analyzed immediately, specimens may be stored at 2-8°C for up to 8 days. If specimens need to be stored for longer than 8 days, they may be stored frozen at -20°C for 30 days.

Reagent 1

Procedure (Automated Analyzers):
Below is a general example of the Estrom LDL - Cholesterol assay procedure for a two reagent automated analyzer.

Estrom LDL-Cholesterol reagent is intended for measurment of serum LDL-Cholesterol using a clinical chemistry analyzer. Below is the best suited test procedure for many of the major analyzers.
This procedure can be adapted and modified for use with other analyzers also.



Abs Difference in absorbance between absorbance 2 & 1
Calibrator : Estrom LDL-C Calibrator
Blanking with : Reagent Blank

4 µl of sample + 300 µl of Reagent 1
↓ 37°C for 5 Minutes
Measure absorbance 1 Bichromatically at 660/546 nm
↓
Add 100 µl of Reagent 2
↓ 37°C for 5 Minutes
Measure absorbance 2 Bichromatically at 660/546 nm
↓
Calculate LDL-C concentration by using △Abs*

Test Procedure for Semi Auto Analyzers Monochromatic 546 nms
(3 Parts R1+ 1 Part R2)

SYSTEM PARAMETERS:

Reaction type	:	**End Point**
Flow cell Temp	:	**37°C**
Units	:	**mg/dl**
Reagent Volume	:	**0.8 ml (0.6 ml R1, 0.2 ml R2)**
Sample Volume	:	**8 µl**
Wavelength	:	**546 nm**
Blank	:	**Reagent**
Calibrator Conc	:	**Concentration on Vial Lable**
Linearity	:	**400**

Pipette the reagents in to tubes labeled as below.

Reagent	B	C	T
R1	0.6 ml	0.6 ml	0.6 ml
LDL- Calibrator (Concentration on Lable)	----	8 µl	----
Specimen	----	----	8 µl
Mix and incubate for 5 minutes at 37°C			
R2	0.2 ml	0.2 ml	0.2 ml
Mix and incubate for 5 minutes at 37°C			

Mix and read absorbance of Calibrator (C) and Test (T) against Reagent Blank (B) at 546 nm

$$\text{LDL-C Concentration (mg/dl)} = \frac{\text{Abs of T}}{\text{Abs of C}} \times \text{calibrator Concentration}$$

Expected Values:
It is recommended that each laboratory should verify the reference interval for its patient population.

Estrom LDL Cholesterol Classification:

<130 mg/dL	Desirable
130-159 mg/dL	Borderline High Risk
>160-189 mg/dL	High Risk
>190 mg/dL	Very High Risk
<100 mg/dL	Optimal
100-129 mg/dL	Near Optimal/Above Optimal

Measuring Range : From detection limit of 6.7 mg/dl to linearity limit of 400 mg/dl If the result obtained is greater than linearity limit, dilute the sample 1/2 with NaCl (9 g/L) and multiply the result by 2.

Results:
To convert from conventional units to S.I. units, multiply the conventional units by 0.02586.
mg/dL x 0.02586 = mMol/L LDL-cholesterol

References:
1. Gotto AM, Lipoprotein metabolism and the etiology of hyperlipidemia, Hospital Practice, 23: Suppl.1, 4 (1988).

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	Attention,see instructions for use		Consult Instructions For Use
IVD	For in vitro diagnostic use only	REF	Catalog #
2°C 8°C	Store between 2-8°C	LOT	Lot Number
	Do not use if package is damaged		Date of Manufacturing
	Manufacturer		Use by

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